Caenorhabditis elegans (Nematoda) and Hypoaspis aculeifer (Acari) as new ecotoxicological tools for the assessment of organic materials used in agriculture

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Caenorhabditis elegans (Nematoda) and *Hypoaspis aculeifer* (Acari) as new ecotoxicological tools for the assessment of organic materials used in agriculture

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Abstract

The modern society is always producing more and more wastes. Some of them, mainly organic ones, can be valued to be in accordance with sustainable practices. Among organic wastes, agricultural, municipal and industrial organic materials (AMIOM) are intended to be used in agriculture. According to the numerous origins of these materials, the input of undesirable pollutants can't be avoided. Therefore it has been found necessary to assess their potential deleterious effects on the environment prior to their use. For that purpose, ecotoxicological characterization of different AMIOM was set up using routine terrestrial and aquatic bio-assays. In addition, two new model organisms (the nematode *Caenorhabditis elegans* and the predaceous mite *Hypoaspis aculeifer*) were included in the test batteries in order to conclude on their relevance in comparison with routine tests. Further work should allow defining a battery of bio-assays for the *a priori* hazard assessment of AMIOM.

Introduction

In the global context of environmental sustainability, there is an increase of the use of agricultural, municipal and industrial organic materials (AMIOM) in agriculture. These materials are useful to supply nutrients for crops and/or to improve soil physical characteristics. However, AMIOM can be a significant source of pollutants [1] depending on their origin or production process. Soil quality is of importance, as soil provides key functions in terrestrial ecosystems like decomposition processes, nutrients and energy flows, which are performed by micro-organisms and invertebrates. These crucial functions explain the interest of protecting the quality of soils, which is included in European environmental policy [2]. That's why, in addition to physico-chemical characterization, there is a need to assess the potential deleterious effects of AMIOM on the environment prior to their use.

Currently, ecotoxicological bio-assays are used to characterize hazards of chemicals and complex matrices on the environment. In this work, the possibility of including two new model organisms (the nematode *Caenorhabditis elegans* and the mite *Hypoaspis aculeifer*) in bio-assays test batteries for AMIOM hazard assessment was studied. *C. elegans*, which is a well-known organism and easy to culture, was selected due to the possibility of testing aqueous as well as solid samples. For the soil mite *H. aculeifer*, its predaceous function was considered as complementary in term of trophic chain compared to the organisms used in terrestrial tests.

Materials and method

AMIOM and test strategy

The AMIOM studied were: limed sewage sludge, ashes (from wastewater sludge, treated wood, paper industry waste and plant residues), deinking sludge, composted household wastes and cow manure (characteristics in Table 1). The test strategy, including terrestrial and aquatic bio-assays, is summarized in Figure 1. Leachates of the mixtures were realized on each dose of each AMIOM. The doses tested were ranging from one to one-hundred times their respective application rates.

Protocol for mixtures preparation, leaching and bio-assays

For soil bio-assays, solid mixtures were prepared with artificial soil, excepted for *C. elegans* for which the soil used for mixtures was an agricultural soil (LUFA 2.2). Five days prior to leaching, mixtures were moistened up to 20% (d.w.) to reach equilibrium. Eluates were obtained after addition of deionised water to the mixtures (ratio 1:10 w:v), which were put under rotating agitation during 24h. Liquid and solid phases were then separated by centrifugation (30min, 2500*g*) and the supernatants were tested for their ecotoxicity toward aquatic organisms. For aquatic tests, samples were filtered at 0.45µm excepted for nematode and daphnid tests, for which the supernatants were directly used after centrifugation. These bio-assays were performed following their respective current normative protocol [3-12], excepted for *C. elegans* terrestrial tests for which the standard protocol was modified for complex matrices testing [13].

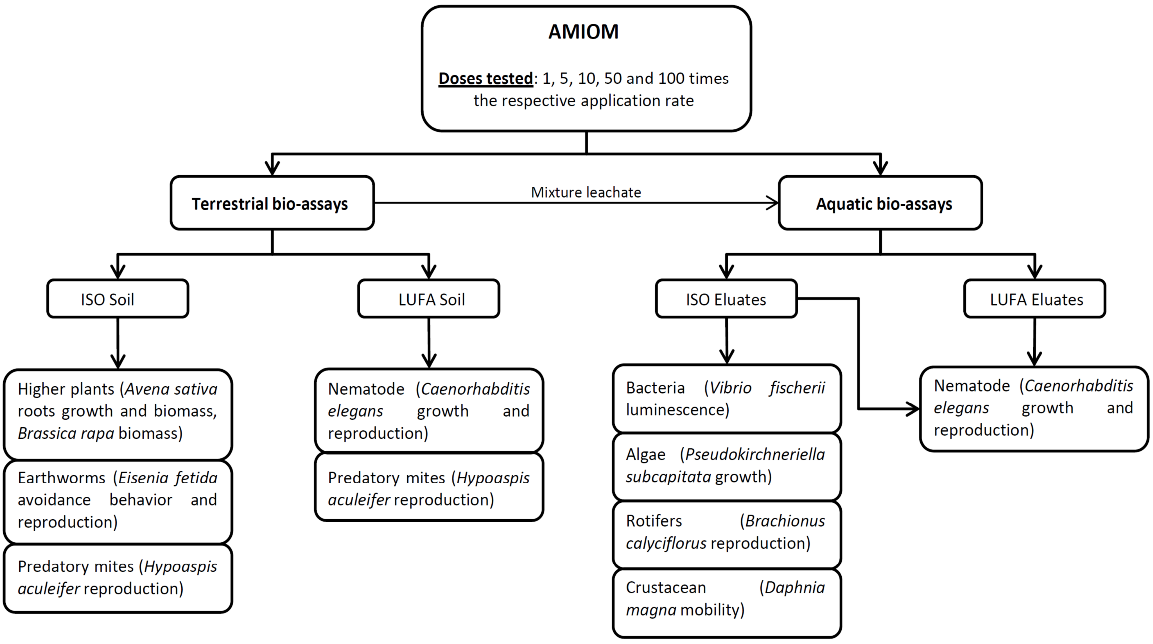


Figure 1: Test strategy for AMIOM testing

Table 1: Physico-chemical properties of AMIOM.
Concentrations are expressed on a dry weight basis.

	Limed sewage sludge	Ashes	Deinking sludge	Composted wastes	Cow manure
Application rate ($T.ha^{-1}$)	3	3	5	3	10
pH	9.3	12.6	8.3	7.3	8.6
WHC (%)	300	50	100	175	290
N total ($g.kg^{-1}$)	9.4	0.8	3.4	14.6	18.5
Organic carbon ($g.kg^{-1}$)	277	< 1	163	319	433
P_2O_5 total ($g.kg^{-1}$)	36.6	8.3	1.9	7.6	8.5
K_2O total ($g.kg^{-1}$)	2.6	8.1	0.7	12.2	33.3
CaO ($g.kg^{-1}$)	148	507	403	60	18.7
MgO ($g.kg^{-1}$)	7	24.1	4.2	5.9	6.9
As ($mg.kg^{-1}$)	4.6	10.1	0.4	3.9	0.4
Cd ($mg.kg^{-1}$)	1.7	3.3	0.1	0.7	0.1
Cu ($mg.kg^{-1}$)	433	383	79.7	96.8	17
Hg ($mg.kg^{-1}$)	0.5	0.6	< 0.01	0.2	0.03
Ni ($mg.kg^{-1}$)	17.8	42.3	1.9	26.1	2.7
Pb ($mg.kg^{-1}$)	39.3	492	3.3	76.8	< 2
Zn ($mg.kg^{-1}$)	458	1549	25.1	395	65.7
∑ 7 PCB ($mg.kg^{-1}$)	< 0.07	< 0.07	< 0.07	< 0.14	< 0.07

Statistical analyses

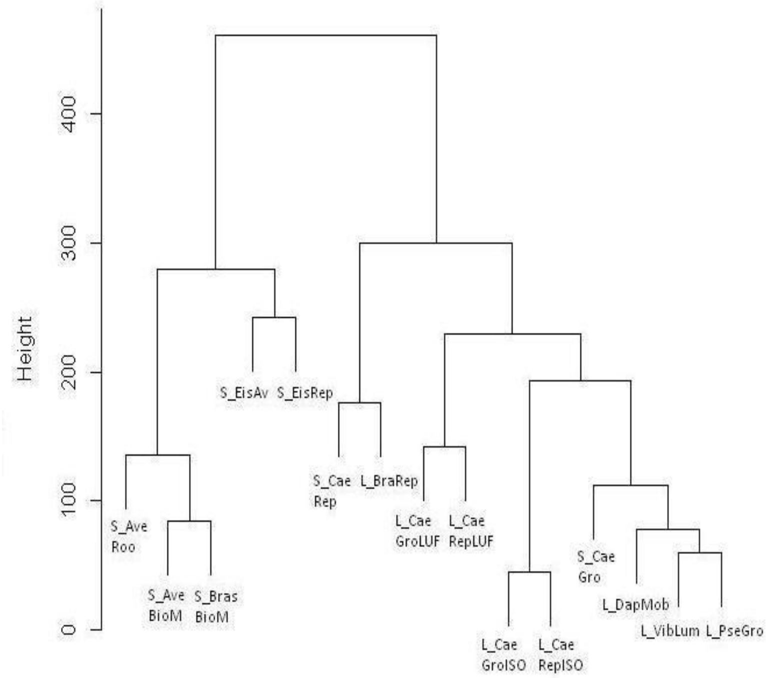
Mean percentage of effect of each test was compared to their respective significant biological percentage of effect, which was based on the ISO 17616 [14] and estimated for *C. elegans*. Then, they were classified as “hormesis effect”, “inhibitory effect” and “no effect”, depending on the responses observed.

In order to determine the response similarity between bio-assays, hierarchical cluster analyses (HCA) were set up using R software [15]. For that purpose, the data used were transformed in mean percentage of effect for each test and AMIOM dose. These data were then changed into a dissimilarity matrix using Euclidian distances, which were agglomerated and ranked with Ward method.

Results

The overview of the data collected to date showed that, for both aquatic and terrestrial bio-assays, the tested AMIOM were not ecotoxic at their respective application rates. Moreover, terrestrial bio-assays showed a higher sensitivity than aquatic ones.

Among higher plants endpoints, the root elongation test was the most sensitive endpoint. Significant effects were recorded for five times the application rate for limed sewage sludge, ten times the application rate for ashes and composted wastes, and fifty times the application rate of cow manure. Significant effects on biomass were recorded generally at 50 times the respective application rate of the materials (deinking sludge had significant effect from five times its application rate). Concerning earthworms, avoidance behaviour was the most sensitive endpoint (significant effects recorded from fifty times the application rate for limed sewage sludge, composted wastes and ashes). However, both avoidance and reproduction endpoints were correlated (Figure 2). Moreover, they were grouped with the plants ones (S_AveRoo, S_AveBioM and S_BrasBioM), which separate terrestrial organism responses from aquatic organisms. For nematode growth and reproduction in terrestrial tests, significant effects were found from fifty times the application rate of ashes and for one-hundred times the application rate of limed sewage sludge and deinking sludge. However, the data of *C. elegans* in soil (S_CaeRep and S_CaeGro) were ranked with aquatic tests. It should also be mentioned that some AMIOM had hormesis effects at over-application rates. This unusual ecotoxic response could be ascribed to intrinsic confounding factors, such as the presence of additional food resources (*i.e.* nutrients, bacteria, growth factors...). Data acquisition concerning *H. aculeifer* reproduction endpoint is still in progress.



Legend :

S_AveRoo : *Avena sativa* roots growth
S_AveBioM: *Avena sativa* biomass
S_BrasBioM: *Brassica rapa* biomass
S_EisAv: *Eisenia fetida* avoidance behavior
S_EisRep: *Eisenia fetida* reproduction
S_CaeGro: *Caenorhabditis elegans* growth (terrestrial test)
S_CaeRep: *Caenorhabditis elegans* reproduction (terrestrial test)
L_PseGro: *Pseudokirchneriella subcapitata* growth
L_VibLum: *Vibrio fischeri* luminescence
L_DapMob: *Daphnia magna* mobility
L_BraRep: *Brachionus calyciflorus* reproduction
L_CaeGroISO: *Caenorhabditis elegans* growth (ISO eluates)
L_CaeRepISO: *Caenorhabditis elegans* reproduction (ISO eluates)
L_CaeGroLUF: *Caenorhabditis elegans* growth

Figure 2: Hierarchical cluster analysis of the bio-assays responses for the AMIOM assessment (Method: Ward, Distance: Euclidian)

Among aquatic bio-assays, the algae *P. subcapitata* endpoint was found to be the most sensitive. The HCA (Figure 2) demonstrated that this bio-assay response was similar to those of the bacteria *V. fischeri* (L_VibLum and L_PseGro). Focusing on *C. elegans* data for both terrestrial and aquatic tests, the reproduction endpoint was found to be more sensitive than the growth. The cluster analysis showed that the nematode responses in aqueous phase (L_CaeGroISO, L_CaeRepISO, L_CaeGroLUF and L_CaeRepLUF) were different depending on the soil used for the mixtures (*i.e.* ISO and LUFA soil).

Conclusion and perspectives

These preliminary results showed that there was a limited redundancy between routine terrestrial and aquatic tests. Moreover, nematode responses for both environmental compartment were ranked nearly altogether and apart from the other tests. This indicates that this species could be an interesting tool to complement the battery of bio-assays.

Further work should allow defining terrestrial and aquatic batteries of bio-assays for the *a priori* hazard assessment of AMIOM, as well as concluding on the interest of the two new model organisms for the AMIOM ecotoxicity study.

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